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Brief Research Communication

No Association Between Two Polymorphisms of the Serotonin Transporter Gene and Combined Type Attention Deficit Hyperactivity Disorder

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Several independent studies have reported association between serotonin transporter gene (SLC6A4) polymorphisms and attention deficit hyperactivity disorder (ADHD). Five studies found evidence for association between the long-allele of a 44-bp insertion/deletion polymorphism (5-HTTLPR) and ADHD. Another two studies corroborated this finding while a further six studies did not find such an association. For a second polymorphism within the gene, a variable number tandem repeat (VNTR) within intron 2, one study demonstrated that the 12/12 genotype was significantly less frequent in ADHD cases compared to controls, while a second study found that the 12-allele was preferentially transmitted to offspring affected with ADHD. To provide further clarification of the reported associations, we investigated the association of these two markers with ADHD in a sample of 1,020 families with 1,166 combined type ADHD cases for the International Multi-Centre ADHD Genetics project, using the Transmission Disequilibrium Test. Given the large body of work supporting the association of the promoter polymorphism and mood disorders, we further

analyzed the group of subjects with ADHD plus mood disorder separately. No association was found between either of the two markers and ADHD in our large multisite study or with depression within the sample of ADHD cases.

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KEY WORDS: attention deficit hyperactivity disorder; serotonin transporter gene; SLC6A4; association study

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The human serotonin transporter gene (SLC6A4, formerly known as 5-HTT) is located at chromosome 17q11.2-12. Two common polymorphisms within the gene have been described: a 44-bp insertion/deletion in the promoter region (5-HTTLPR) [Heils et al., 1996] and a variable number tandem repeat in intron 2 (STin2-VNTR) [Lesch et al., 1994]. Several research groups have studied the association between these SLC6A4 polymorphisms and attention deficit hyperactivity disorder (ADHD). Five studies found evidence for association between the long-allele of the 5-HTTLPR polymorphism and ADHD or

closely related phenotypes [Seeger et al., 2001; Kent et al., 2002; Retz et al., 2002; Beitchman et al., 2003; Curran et al., 2005]. In accord with these data two other studies found that short/short genotype was significantly underrepresented in children with ADHD compared to controls [Manor et al., 2001; Zoroğlu et al., 2002]. However, Li et al. [2007] reported a preferential transmission of the short-allele to ADHD offspring in a Chinese sample and six further studies did not find any association between the polymorphism and ADHD [Langley et al., 2003; Kim et al., 2005; Xu et al., 2005; Banerjee et al., 2006; Wigg et al., 2006; Heiser et al., 2007]. A study that combined the available data by pooling odds ratios across studies found a small but significant association of the long allele of 5-HTTLPR with ADHD [Faraone et al., 2005].

For the STin2-VNTR marker one study found that the 12/12 genotype was significantly less frequent in ADHD cases compared to controls [Zoroğlu et al., 2002], while another study found that the 12-repeat allele was preferentially over-transmitted to ADHD offspring [Banerjee et al., 2006]. A further seven studies did not demonstrate an association between STin2-VNTR and ADHD [Kent et al., 2002; Langley et al., 2003; Curran et al., 2005; Kim et al., 2005; Xu et al., 2005; Heiser et al., 2007; Li et al., 2007].

The aim of this study was to further investigate the relationship between these two polymorphisms of SLC6A4 (in particular the association with 5-HTTLPR that was found to be associated with ADHD following meta-analysis) using a large sample of combined type ADHD cases collected by the International Multi-Centre ADHD Genetics (IMAGE) project. In addition because there is strong evidence that short allele of 5-HTTLPR is associated with symptoms of depression, probably by modifying environmental risks [Furlong et al., 1998; Canli and Lesch, 2007], we further investigated whether the short allele was over-transmitted to the group of children with ADHD comorbid with significant mood symptoms. The hypothesis tested being that aspects of ADHD would act as an *environmental stressor*, increasing the chances of developing depressive symptoms in the presence of the short allele of 5-HTTLPR.

European Caucasian subjects were recruited from 11 specialist clinics in 8 countries: Belgium, Germany, Holland, Ireland, Israel, Spain, Switzerland, and United Kingdom. Ethical approval for this study was obtained from National Institute of Health registered ethical review boards for each center. Detailed information sheets were provided and informed consent obtained from children and their parents. All ADHD probands and their siblings were aged 5–17 at the time of entry into the study and access was required to one or both biological parents for DNA collection. Entry criteria for probands were a clinical diagnosis of DSM-IV combined subtype ADHD, having one or more full siblings available for ascertainment of clinical information and DNA collection. Exclusion criteria applying to both probands and siblings include autism, epilepsy, IQ < 70, brain disorders and any genetic or medical disorder associated with externalizing behaviors that might mimic ADHD.

All raw data were centralized and stored on a secure database at the MRC Social Genetic Developmental Psychiatry research center in London. The diagnosis of ADHD was made following a parent interview with the Parental Account of Child Symptoms interview [PACS; Taylor et al., 1986] that asks about ADHD symptoms in various settings. An algorithm was used to derive each of the DSM-IV ADHD symptoms from the PACS interview data and these were combined with items that scored two or more from teacher ratings of DSM-IV items taken from the long version of the Conners' Teacher Rating Scale [Conners, 1995]. The diagnosis of ADHD was made if sufficient items were identified to fulfill DSM-IV criteria, and both impairment (based on severity of symptoms identified in the PACS interview) and pervasiveness (based on the presence of ADHD symptoms in more than one setting from PACS and scoring more than one item on the teacher Conners) were present. In a few cases where no Conners data were present pervasiveness was defined on the basis of PACS data alone.

The PACS interview was also used to identify the group of ADHD cases with a possible mood disorder. Although PACS does not formally generate DSM-IV diagnostic categories for mood disorders, it identifies individuals reporting high and impairing levels of mood symptoms. The key criteria for depression were the presence of marked misery lasting more than 6 hr during which time the child could only be cheered up with difficulty, on 3–6 days a week, or the presence of severe misery (could not be cheered up at all), on at least 1 or 2 days a week. Similar frequency criteria applied for other changes in mood that were either marked (showing diminished interest or pleasure in activities, or marked lack of energy and fatigue, lasting 6 hr or more) or severe (no engagement even in normally pleasurable activities).

The final dataset used in this study consisted of 1,020 ADHD families with 1,166 DSM-IV combined type probands. Of these there were 199 ADHD cases (15% of the cases) with evidence of a comorbid mood disorder from the PACS interview data. DNA was available for both parents in 891 families (87%) and from one parent in 129 families (13%). Eighty-eight percentage of the ADHD cases were male.

Genotyping followed routine procedures. The 44-bp insertion/deletion 5-HTTLPR polymorphism and the 17-bp STin2-VNTR polymorphism were genotyped using the methods described in Zoroğlu et al. [2002]. The program suite UNPHASED was used to test the linkage disequilibrium (LD) between the individual marker locus and haplotypes [Dudbridge, 2003]. TDTPhase was used for the transmission disequilibrium test (TDT) analysis from family genotype data.

In total we obtained data from 1,166 affected children for the two polymorphisms located in the SLC6A4 gene. We found no evidence for the preferential transmission of the long-allele of 5-HTTLPR to affected offspring as previously reported, and also no evidence of biased transmission of the short-allele (Table I). For STin2-VNTR, three alleles (9-repeat, 10-repeat, and 12-repeat) were detected in our sample with the most common alleles being the 10-repeat and 12-repeat alleles. The

TABLE I. Results of the TDT Analysis of 5-HTTLPR and STin2-VNTR Polymorphisms

Polymorphism	Allele	Transmitted	Non-transmitted	χ^2	P-value	OR
5-HTTLPR	L	496	462	1.21	0.27	1.07
	S	462	496	1.21	0.27	0.93
STin2-VNTR	9	25	25	0.00	1.00	1.00
	10	462	452	0.11	0.74	1.02
	12	463	473	0.11	0.74	0.98

5-HTTLPR likelihood ratio test: null = -664.0, alternative = -663.4, LRS = 1.207, df = 1, $P = 0.272$.

STin2-VNTR likelihood ratio test: null = -658.5, alternative = -658.4, LRS = 0.110, df = 2, $P = 0.947$.

The degrees of freedom for the allele-specific test is 1.

TABLE II. Results of the TDT Analysis of the Haplotypes for 5-HTTLPR and STin2-VNTR Polymorphisms

Haplotype	Transmitted	Freq-T	Non-transmitted	Freq-NT	χ^2	P-value
L-9	22	0.02	21	0.02	0.02	0.88
L-10	290	0.30	269	0.28	0.62	0.43
L-12	262	0.28	262	0.28	0.00	1.00
S-9	1	0.00	0	0.00	0.00	1.00
S-10	95	0.10	95	0.10	0.00	1.00
S-12	281	0.30	304	0.32	0.68	0.41

T, transmitted; NT, non-transmitted.

Global likelihood ratio test: null = -533.03, alternative = -531.93, LRS = 2.200, df = 5, $P = 0.821$.

common alleles were not significantly associated with ADHD in this sample (Table I).

We also found no evidence of over-transmission of the 5-HTTLPR risk allele for depression, in the sub-group with ADHD plus evidence of a mood disorder ($\chi^2 = 1.9$, $P = 0.17$ for 5-HTTLPR; $\chi^2 = 2.4$). Similarly there was no evidence of association with the intron 2 markers in this group ($P = 0.30$ for STin2-VNTR), nor in the group with ADHD with no evidence of a comorbid mood problem with either marker ($\chi^2 = 0.74$, $P = 0.39$ for 5-HTTLPR; $\chi^2 = 0.05$, $P = 0.97$ for STin2-VNTR). LD between the two polymorphisms was significant but moderate using the D' and r^2 statistic ($D' = 0.42$, $r^2 = 0.29$). There was no evidence of biased transmission of any of the haplotypes (Table II).

In summary, we investigated the relationship between ADHD and two SLC6A4 polymorphisms that had previously been reported to be associated with ADHD. Our large multisite study of 1,166 combined type probands found no evidence for an association with either of the two polymorphisms. Given the large body of work supporting the association of the promoter polymorphism and mood disorders [Canli and Lesch, 2007] we analyzed the group of subjects with ADHD plus mood disorder separately, but also found no evidence for association. Formal meta-analysis of these with other available datasets is now required to clarify whether the serotonin transporter gene has an aetiological role in ADHD susceptibility.

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